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REVIEW ARTICLE

Light dependent protochlorophyllide oxidoreductase: a succinct look

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Abstract

Reducing protochlorophyllide (Pchlide) to chlorophyllide (Chlide) is a major regulatory step in the chlorophyll biosynthesis pathway. This reaction is catalyzed by light-dependent protochlorophyllide oxidoreductase (LPOR) in oxygenic phototrophs, particularly angiosperms. LPOR-NADPH and Pchlide form a ternary complex to be efficiently photo-transformed to synthesize Chlide and, subsequently, chlorophyll during the transition from skotomorphogenesis to photomorphogenesis. Besides lipids, carotenoids and poly-cis xanthophylls influence the formation of the photoactive LPOR complexes and the PLBs. The crystal structure of LPOR reveals evolutionarily conserved cysteine residues implicated in the Pchlide binding and catalysis around the active site. Different isoforms of LPOR viz PORA, PORB, and PORC expressed at different stages of chloroplast development play a photoprotective role by quickly transforming the photosensitive Pchlide to Chlide. Non-photo-transformed Pchlide acts as a photosensitizer to generate singlet oxygen that causes oxidative stress and cell death. Therefore, different isoforms of LPOR have evolved and differentially expressed during plant development to protect plants from photodamage and thus play a pivotal role during photomorphogenesis. This review brings out the salient features of LPOR structure, structure–function relationships, and ultra-fast photo transformation of Pchlide to Chlide by oligomeric and polymeric forms of LPOR.

Keywords Chlorophyll biosynthesis · Light-dependent protochlorophyllide oxidoreductase · Prolamellar body · Photooxidative damage

Introduction

Protochlorophyllide oxidoreductase (POR) is a key enzyme within the chlorophyll biosynthesis pathway that is involved in the reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide a). POR exists in two different non-homologous enzymatic forms (1) NADPH Light dependent Protochlorophyllide Oxidoreductase (LPOR) and (2) Light Independent or Dark Operative Protochlorophyllide Oxidoreductase (DPOR/LIPOR). LIPOR is chloroplast encoded hetero-octameric complex present in anoxygenic prokaryotes, oxygenic cyanobacteria, several bryophytes, pteridophytes and gymnosperms that does not have an absolute requirement of light for catalysis. LPOR is nuclear encoded, single polypeptide of approx. 36 kda that is post-translationally imported to plastids (Armstrong et al. 1995; Fujita 1996; Gabruk and Mysliwa-Kurdziel 2020). Light is indispensable for the activity of LPOR enzyme much like the DNA repair enzyme DNA photolyase (Begley 1994; Björn 2018), bacterial chlorophyllide a reductase (COR) (Saphier et al. 2005), cyanobacterial chlorophyllide f synthase (Chen et al. 2010; Ho et al. 2016) and fatty acid photodecarboxylase (FAP) (Sorigué et al. 2017). Unlike archegoniate, LPOR is the principal Pchlide reducing enzyme in angiosperms. In addition to light and Pchlide as a target substrate, LPOR requires NADPH as a reductant to catalyse the stereospecific reduction of the C17- C18 double bond of (Pchlide a)—to (Childe-a) (Griffiths 1974; Schoefs and Franck 2003).

Photoreduction of Pchlide to Chlide is an ultrafast event that involves transient charge separation across the C17-C18 double bond of the pigment Pchlide leading to the formation of charge transfer intermediates which facilitate the step wise hydride and proton transfer (Archipowa et al. 2018).

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These intermediates have been analysed on an ultra-fast time scale by time resolved fluorescent measurements (Heyes et al. 2003; Sytina et al. 2008).

LPOR a short-chain dehydrogenases/reductases superfamily confrère

LPOR belongs to a large family of enzymes known as short-chain dehydrogenases/reductases (SDRs) (Yang and Cheng 2004; Wilks and Timko 1995; Moummou et al. 2012). SDR is part of a large superfamily of enzymes known as the 'RED' (Reductases, Epimerases, Dehydrogenases) that catalyze a variety of NADP (H)—or NAD(P) + -dependent reactions (Wilks and Timko 1995; Oppermann et al. 2003; Moummou et al. 2012) involving hydride and proton transfer (Hoeven et al. 2016; Archipowa et al. 2018). This is one of the oldest and most diverse protein families present in prokaryotes and eukaryotes that typically occur as oligomers (Oppermann et al. 2003; Yang and Cheng 2004). It has a wide range of substrates involved in secondary metabolic routes ranging from polyols, retinoids, sterols, sugars, aromatic compounds, and xenobiotics (Persson et al. 2003). Plant LPORs are assigned to SDR73C family in the SDR superfamily (Dong et al. 2020).

The classical SDR family of proteins containing all oxidoreductases has two domains, one for binding of the cofactor and another for binding the substrate (Moummou et al. 2012). Despite the considerably low sequence similarity (15–30%), SDR family members bear significant structural similarity such as a common a/ß folding pattern with Rossmann fold and a highly conserved active site containing YxxxK residues in the catalytic motif (YKDSK in LPOR) that participate in the proper coordination with NADPH and Pchlide binding (Lebedev et al. 2001; Gabruk et al. 2016). The N terminal contains a conserved glycine-rich motif (Gly-X-X-X-Gly-X-Gly) in SDR and GASSGV/LG in all LPORs. This is important for structural integrity and binding of the pyrophosphate portion with NADPH (Dong et al. 2020). A key feature of the SDR superfamily is its catalytically important tetrad Ser-Asn-Tyr-Lys for proton transfer and stabilization of reaction intermediates. The catalytic triad in POR contains Thr 145 instead of Ser residue (Moummou et al. 2012; Dong et al. 2020). Site-directed mutagenesis and in vivo analysis confirm that Tyr and Lys are the most conserved at the catalytic site in all LPOR members and these are indispensable for the enzymatic catalytic activity (Wilks and Timko 1995; Suzuki and Bauer 1995; Lebedev et al. 2001; Heyes and Hunter 2002). Two mechanisms of photochemical activation of Pchlide were proposed. A) Tyr residue acts as a general acid upon deprotonation and facilitates hydride transfer to or from NAD (P) + /H (Lebedev et al. 2001) to C17 of Pchlide that facilitates a proton transfer at the C-18 position (Johannissen et al. 2022). B) Alternatively, the hydride transfer reaction is shown to occur in a stepwise manner involving an initial electron transfer from NADPH to the excited state of Pchlide followed by proton transfer from a tyrosine reside to C18 and immediately followed by hydride transfer from NADPH to C17 (Heyes and Hunter 2005; Archipowa et al. 2018; Kim et al. 2021).

The mutation of either Tyr 275 or Lys-279 does not completely abolish the catalytic activity of LPOR. However, mutation of either residue impairs the formation of the ground state ternary enzyme–substrate complex, indicating their key role in substrate binding (Dahlin et al. 1999; Heyes and Hunter 2002; Heyes et al. 2021). Both residues have multiple roles in catalysis, involving the formation of the ground state ternary enzyme–substrate complex, stabilization of a Pchlide excited state species, and proton transfer to the reaction intermediate formed after the light reaction (Menon et al. 2009; Dong et al. 2020) (Fig. 1).

LPOR contains 14 amino acids unique TFT domain that distinguishes LPOR from other structurally related SDR enzymes (Gabruk et al. 2012). The LPOR homologs are structurally conserved with sequence identities of about 54%—65% between higher plant, cyanobacterial and algal enzymes (Suzuki and Bauer 1995; Li and Timko 1996; Dahlin et al. 1999). The secondary structure analysis of LPOR by CD spectroscopy shows 33% alpha-helix, 19% beta-sheets, 20% turn, and 28% random coil (Birve et al. 1996).

Crystal structure of LPOR

Crystal structure of LPORs in their free form (Zhang et al. 2019) and complexed with NADPH have been solved from *Thermosynechococcus elongatus* and *Synechocystis* sp. PCC 6803 at 1.3–2.4 Å resolution (Zhang et al. 2019; Dong et al. 2020). The above studies highlight the potential importance of hydrogen-bonding networks involving the interaction of LPOR active site residues and Pchlide. The general scaffold of protein remains similar to the typical αβα-topology with a central β-sheet and multiple flexible loops. The crystallographic studies of LPOR demonstrate an 8β-sheet consisting of strands β 3-β 2- β 1- β 4- β 5- β 6- β 7- β 8, the latter being antiparallel. The β-sheets are surrounded by 6 α-helices, (αA, α B, α H) on one side and (αC, α D, α F) on the other side (Dong et al. 2020). According to Zhang et al. (2019) Pchlide binds to the LPOR active site by orientation of the polar functional groups that form hydrogen bonds with hydrophilic residues in the deep binding pocket of enzyme. The hydrophobic Pchlide residue interacts with hydrophobic LPOR residues to form a hydrophobic patch on the surface of the protein. The pigment-bound AtPORB

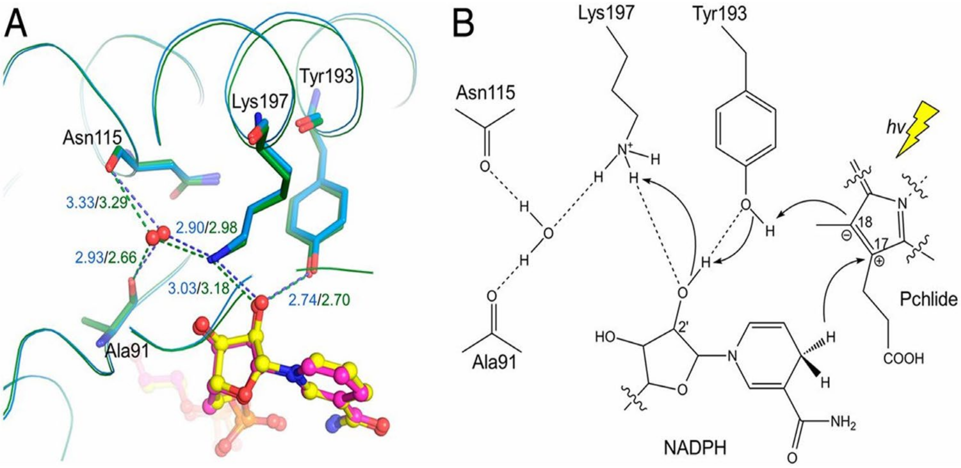

Fig. 1 Proposed proton-relay path from Dong et al. (2020). **A** The hydrogen bond network bridging the Tyr193 ηO and a solvent water molecule within the SyLPOR and TeLPOR structures. The well-positioned water, shown in the red sphere, is fixed by the backbone oxygens of Ala91 and Asn115, and the ε-amino group of Lys197. The hydrogen bonds are shown in dashed lines and the bond lengths (Å) are in blue for SyLPOR and dark green for TeLPOR. **B** A proposed proton-relay path following the hydride transfer from NADPH to C17. The photon energy (hv) is represented by a yellow thunderbolt (Dong et al. 2020)

oligomers form helical filaments and remain embedded in the outer leaflet of the lipid bilayer. This shapes the architecture of photosynthetic membranes by forming highly curved PLBs (Nguyen et al. 2021a, b).

The LPOR homologs of *Synecocystis* and *T. elongatus* contain four evolutionarily conserved cysteine residues; Cys38, Cys89, Cys199, and Cys226 around the active site (Silva 2014; Dong et al. 2020). Cys 226 in the loop between β6 and αG is essential for LPOR membrane interaction. The conserved active site residue Tyr previously touted as the proton donor is thought to be important for Pchlide binding. Site-directed mutagenesis studies in *T. elongatus* and LPOR ternary structural model by Zhang et al. (2019) reveal that cys226, located close to C18 of Pchlide, plays a crucial role in Pchlide binding and hydride transfer. Cys226 may act as a proton donor either directly or via the water-mediated network. Pchlide also interacts with Tyr223 and Gln248 active site residues in *T. elongatus* during LPOR photochemistry. Thus, the proton relay pathway takes place by abundant intermolecular polar interactions among NADPH, LPOR, and surrounding water molecules with the help of functional groups and backbone atoms to stabilize the cofactor (Dong et al. 2020) Fig. 2.

Near the nicotinamide end, a clam-shaped cavity is formed by predominantly hydrophobic and aromatic residues consisting of Leu232, Phe233, His236, Tyr237, Phe240, Phe243, and Phe246 etc. (Dong et al. 2020). The extra loop of 33 amino acid segments uniquely present in LPOR and absent in other SDR enzyme superfamily members overlap with certain fragments of the clam–shaped cavity. The orientation of Pchlide within the binding cavity is essential for the enzyme reaction mechanism (Pesara et al. 2023). It participates in Pchlide binding, formation of pigment-complexed POR aggregates and Chlide release (Birve et al. 1996; Reinbothe et al. 2003; Sameer et al. 2021).

The LPOR oligomerization takes place upon Pchlide binding which brings about the interaction of the hydrophobic residues and intermolecular interactions in the two distally located lid regions in the POR monomer active site (Gabruk and Mysliwa-Kurdziel 2015; Zhang et al. 2019, 2021). A POR octamer has been isolated and its structure investigated by cryo-electron microscopy at 7.7 Å resolution. This structure shows that oligomer formation is most likely driven by the interaction of amino acid residues in the highly conserved lid regions (Zhang et al. 2021). In closed conformation two short flexible alpha helices act as lid to cover the hydrophobic edge of Pchlide in *T. elongates*. However, only one longer alpha helix is observed in *Synecocystis* with an additional loop that extends from the central beta sheet. The lid region positions the Pchlide optimally for photocatalysis and its movement triggers large conformational changes that facilitates LPOR oligomer formation (Zhang et al.2021). According to Zhang et al. (2019) three flexible regions (residues 146–160, 228–255 and 284–291) are missing in *T. elongatus* but present in coenzyme bound *Synecocystis* LPOR. These highly ordered regions are implicated in NADPH binding to LPOR (Zhang et al. 2019).

LPOR isoforms

LPOR contains multiple isoforms that exhibit differential subcellular localization, expression pattern, mRNA stability, plastid import pathway and response to light. Although

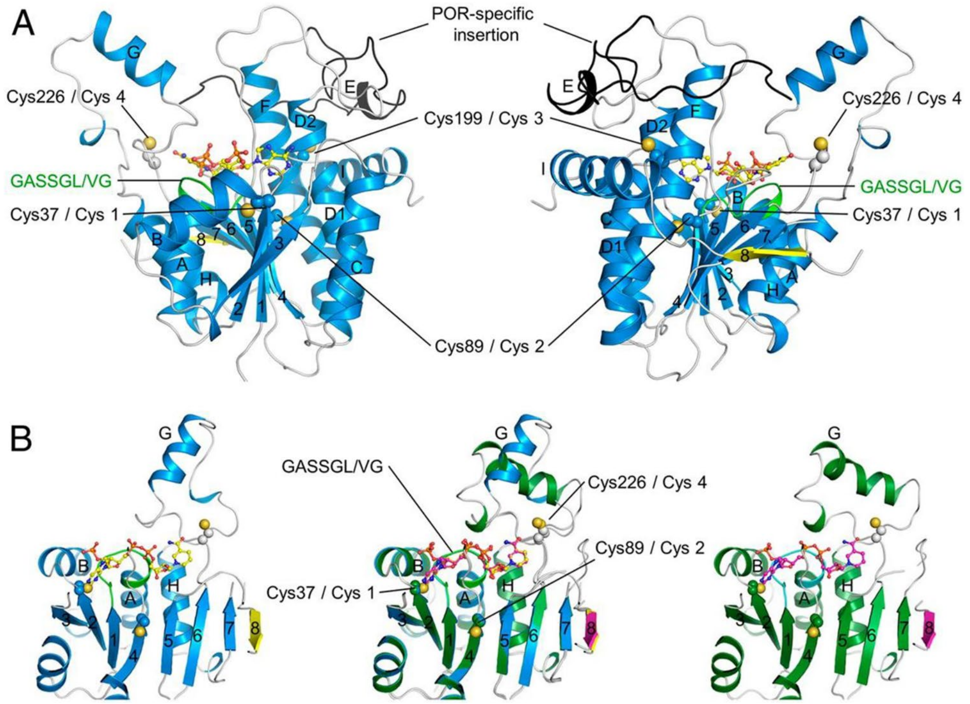

Fig. 2 The crystal structure of SyLPOR and TeLPOR from Dong et al. (2020). Ribbon representation of the overall structures of SyLPOR and TeLPOR. **A** Two side views of SyLPOR. The secondary structure elements are colored in blue except the antiparallel β8 in yellow. The loop region is in gray. The LPOR-specific insertion is colored in black. The NADPH-binding sequence is colored in green. Four cysteine residues are shown in sphere mode. The cofactor NADPH is shown in stick-and-ball mode. **B** Front view of SyLPOR (Left), TeLPOR (Right), and their superimposition (Middle). The secondary structure elements of TeLPOR are colored in deep green except β8 in magenta; the NADPH-binding sequence is colored in cyan. The α-helices are labeled alphabetically, and the β-strands are labeled numerically (Dong et al. 2020)

LPOR proteins were known since a long time, the genes coding LPORA and LPORB were first identified *in A. thaliana* and *H. vulgare* (Reinbothe et al. 1996). Since then, LPOR sequences have been discovered in a number of phototrophs. In higher plant LPOR isoforms show > 70% sequence identity for the precursor polypeptides and > 80% sequence identity for the mature proteins. The transit peptide region at the N terminal which is not a part of the mature enzyme shows lowest homology (Dong et al. 2020).

In gymnosperms LPOR is encoded by a large multigene family, for instance eleven copies of PORB and two copies of PORA have been identified in (Loblolly pine) *Pinus tadea*, *Pinus mungo, Pinus strobus* (Spano et al. 1992; Forreiter and Apel 1993; Skinner and Timko 1998, 1999). *A. thaliana* contains three LPOR isoforms (*Arabidopsis thaliana* PORA (AtPORA), AtPORB, and AtPORC) (Reinbothe et al. 2010; Sousa et al. 2013; Masuda and Takamiya 2004; Oosawa et al. 2000; Benli et al. 1991; Armstrong et al. 1995; Su et al. 2001; Pattanayak and Tripathy 2002; Nguyen et al. 2021a, b). *Zea mays* contains PORA and two PORB orthologs PORB1 and PORB2, latter promoting tocopherol biosynthesis post anthesis. Increase in tocopherol content was likely accomplished by increased turnover of Chls that supply phytol the precursor for tocopherol biosynthesis (Zhan et al. 2019).

Two POR isoforms are found in *Nicotiana tabacum* (Masuda and Takamiya 2004), *Lycopersicon esculentum* (Masuda and Takamiya 2004), *Zea mays* (Horton and Leech 1975), *Oryza sativa* (Sakuraba et al. 2013; Kwon et al. 2017), *Hordeum vulgare* (Apel et al. 1980; Apel 1981; Schulz et al. 1989; Holtorf et al. 1995), ornamental plant *Amaranthus tricolor* (Iwamoto et al. 2001) and several other species. A single LPOR gene has been detected in *Synechocystis* sp.strain PCC6803 (Suzuki and Bauer 1995; Fujita et al. 1998; Rowe and Griffiths 1995; Kaneko et al. 1996), *Plectonema boryanum* (Fujita et al. 1998), *Phormidium lamonosum* (Fujita et al. 1998; Rowe and Griffiths 1995), *Chlamydomonas reinhardtii* (Li and Timko 1996), *Marchantia paleacea* (Takio et al. 1998), *Pisum sativum* (Spano et al. 1992), *Triticum aestivum* (Teakle and Griffiths 1993; Masuda and Takamiya 2004; Schoefs and Franck 2003), *Avena sativa* (Darrah et al. 1990; Klement et al. 1999), *Musa* (Coemans et al. 2005) and *Cucumis sativus* (Yoshida et al. 1995; Fusada et al. 2000). PORA is exclusively expressed in etiolated seedlings and its mRNA abundance and its expression declines rapidly upon illumination in *Hordeum vulgare* and several other species (Armstrong et al. 1995; Holtorf et al. 1995; Reinbothe et al. 1995; Reinbothe and Reinbothe 1996; Runge et al. 1996; Oosawa et al. 2000; Masuda et al. 2003; Garrone et al. 2015). PORA is light-sensitive, it majorly accumulates during skotomorphogenesis and plays a critical role in the etioplast development and photomorphogenesis (Paddock et al. 2012; Gabruk and Mysliwa-Kurdziel 2015). As Pchlide accumulates in dark-grown tissues in large amounts, PORA mainly evolved to ensure fast photo-transformation of Pchlide to Chlide upon illumination to prevent Pchlide-photosensitized and $^1O_2$-induced damage during early stage of seedling greening (Fujii et al. 2017).

Overexpression studies of PORA in porB-1 porC-1 double mutant restore the Chl synthesis at varying light intensities indicating that transiently active PORA might be capable of functioning at a range of light intensities (Paddock et al. 2010). In essence, PORA expression is negatively regulated on exposure to light. In contrast, PORB transcripts are majorly present in thylakoid membranes in young dark-grown seedlings and in illuminated seedlings. PORB concentration remains unaffected during the change of illumination conditions from dark to light (Armstrong et al. 1995; Holtorf et al. 1995; Oosawa et al. 2000; Lebedev and Timko 1999; Ha et al. 2017; Buhr et al. 2017). PORB is present right from the seedling development to throughout the life of the plant in mature tissues. PORB closely resembles PORA but there are significant differences between the two enzymes with respect to gene expression, requirements for import of the precursor into the chloroplast and stability in light. Thus, PORA and PORB have unique functions in etiolated seedlings and at the onset of greening (Aronsson et al. 2000; Masuda et al. 2003; Dahlin et al. 1999; Pattanayak and Tripathy 2002, 2011).

PORC is expressed in a light intensity dependent manner, being highly expressed in high light (Oosawa et al. 2000; Su et al. 2001; Pattanayak and Tripathy 2002). PORC mRNA accumulates only after illumination in etiolated seedlings and is predominantly detected in fully matured green tissues during development and throughout the life of the plant (Su et al. 2001; Pattanayak and Tripathy 2002, 2011; Paddock et al. 2010). Despite the physiological equivalence and a perceived redundancy in PORB and PORC functions in mature plants under normal growth conditions, it has been seen that PORC is differentially regulated and is not under circadian control like PORB. The PORC transcripts are positively regulated by increasing intensity of light while PORB mRNA decreased partially under high light conditions in *Arabidopsis*. Thus, PORB although constitutively active from the seedling stage to the mature plants, it has been observed less active under high light conditions (Masuda et al. 2003). Based on the biochemical analysis, interaction with lipids and evolutionary studies Gabruk and Mysliwa-Kurdziel (2020), proposed two group of LPOR enzymes- a) Lipid independent Z type LPOR—bacterial origin and b) Lipid dependent -Plant origin LPOR- S type (AtPORC type active enzymatically active with and without lipids) and L type LPOR (are active when bound to lipid membrane).

Role of LPOR during greening

When the seed germinates beneath the earth in the absence of light i.e., during skotomorphogenesis two structurally unique lipid-pigment inner membrane systems are present in the etioplasts, prolamellar bodies (PLBs) and prothylakoids (PTs) (Kahn 1968a, b; Ryberg and Sundqvist 1982b; Wellburn 1984; Artus et al. 1992; Gabruk and Mysliwa-Kurdziel 2015). The PLB has a tendency to form highly organised cubic phase non lamellar structures, while PTs form sac like lamellar bilayers (Gunning 1965; Selstam and Sandelius 1984; Brentel et al.1985). The PLBs and PTs predominantly contain galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) upto 80 mol %. The MGDG is dominant in PLBs while DGDG is more dominant in PTs. The anionic lipids sulfosyl quinoline diacylglycerol (SQDG) and phosphatidylglycerol (PG) are present to a lesser extent (upto 20 mol%) in PLB membrane (Ryberg et al. 1983; Selstam 1998; Selstam and Sandelius 1984; Solymosi and Schoefs 2008, 2010; Gabruk et al. 2017; Fujii et al. 2017, 2018; Gabruk and Mysliwa-Kurdziel 2020; Yoshihara and Kobayashi 2022).

LPOR is the most abundant protein in the PLBs while other proteins are dominant in PTs where LPOR is present only in minor amounts (Selstam and Sandelius 1984; Dehesh and Ryberg 1985; Lindsten et al. 1988). In PLBs majority of the LPOR is present in association with the substrate Pchlide and co substrate NADPH (Griffiths 1975; Griffiths et al. 1984; Boddi et al. 1990; Schulz and Senger 1993). These ternary complexes are called as subunits which are further built into macrodomains with regular polymeric structures (Solymosi et al. 2004, 2007). The small aggregates (dimers) are present at the outer surface of the PLBs, and the large aggregates (oligomers) are built into the inner membrane of PLBs (Wiktorsson et al. 1993; Klement et al. 2000).

Numerous studies on leaves and isolated plastids indicate that Pchlide: LPOR: NADPH aggregates interact with the membrane lipids of PLB and are responsible for light triggered PLB dispersion (Engdahl et al. 2001; Aronsson et al. 2008). In vitro studies have shown that PLB lipids, SQDG and PG increases NADPH binding affinity to plant LPOR, while MGDG affects the spectral properties of the complex and may trigger oligomerization (Nguyen et al. 2021a, b). The decrease in DGDG content also resulted in significant structural PLB lattice perturbations, strong reduction of PT number, and retarded PLB disassembly in the light (Gabruk et al. 2017; Fujii et al. 2017, 2018; Gabruk and Mysliwa-Kurdziel 2020). *A thaliana* PG and SQDG single and double mutant analysis shows a partial deficiency in PG biosynthesis loosens the lattice structure of PLBs and impairs the insertion of Mg^{2+} into protoporphyrin IX, leading to a substantial decrease in Pchlide content. Although a complete lack of SQDG biosynthesis does not substantially affect PLB formation and Pchlide biosynthesis, a complete lack of SQDG in addition to partial PG deficiency strongly impairs these processes and affects the dynamics of LPOR complexes after photoconversion of Pchlide to Chlide. These studies make it evident that PG is involved in Pchlide biosynthesis and PLB formation but SQDG likely plays a supplementary role in these processes. This suggests different involvements of PG and SQDG in LPOR complex organization (Yoshihara et al. 2023). The exact mechanisms for these processes, however, are still elusive (Gabruk and Mysliwa-Kurdziel 2020). Prokaryotic LPORs from *Gloeobacter violaceus* PCC7421 and *Synechocystis* sp. PCC6803 could successfully restore characteristic PLB structures in LPORA knockout mutant of *A. thaliana*. Even though the size and structure of PLBs were normal, there was a lower ratio of photoactive to non-photoactive Pchlide (Masuda et al. 2009). LPOR overexpression studies in LIPOR deficient cyanobacterium in the dark show the formation of PLB-like ultra-structures in dark. These studies clearly show the intrinsic capability of LPOR to trigger PLBs formation irrespective of its origin in phototrophs (Yamamoto et al. 2020).

Certain studies indicate that cyanobacterial LPOR operate in a lipid independent manner in contrast to higher phototrophs where galactolipids play an important role in chloroplast differentiation from proplastids or etioplasts (Shipley et al. 1973; Gounaris et al.1983; Solymosi et al. 2007; Gabruk and Mysliwa-Kurdziel 2020). As a result of the light-induced reduction of Pchlide, PLBs disintegrate and the etioplast develops into the chloroplast. The PTs ultimately transform into well-organized thylakoid membranes (Oliver and Griffiths 1982; Lindsten et al. 1988). The isoforms of LPOR are present at different locations of etio-chloroplasts inner membranes (Grzyb et al. 2013; Kowalewska et al. 2016). In *A. thaliana* PORA isoform, amino acid residues 85–88 and 240–270 participate in oligomerization (Gabruk et al. 2016). There is a possibility of the presence of species-specific motifs in plant LPORs within the oligomerization region (Dong et al. 2020).

Besides lipids, carotenoids and poly-cis xanthophylls influence the formation of the photoactive LPOR complexes and the PLBs (Chahdi et al. 1998; Park et al. 2002; Bykowski et al. 2020). In higher plants the carotenoid isomerase (CRTISO) catalyzes the isomerization of poly-cis-carotenoids to all-trans-carotenoids. The absence of PLBs in crtISO (carotenoid isomerase) mutants demonstrates that carotenoids facilitate early chloroplast development during the first critical days of seedling germination and photomorphogenesis (Park et al. 2002). *A. thaliana* seedling deficient in lutein accumulated lower amount of Pchlide compared to wt. in etiolated condition. Thus, indicating an equally important role of photoprotective xanthophyll carotenoids such as lutein in the morphology of the PLB and its interaction with LPOR (Park et al. 2002; Jedynak et al. 2022).

Recently, electron cryo-tomographic studies of pea and maize etioplasts revealed that ATP synthase monomers are enriched in the PTs. The entire tubular lattice is covered by regular helical arrays of LPOR oligomers inserted into the outer leaflet of PLBs (Floris and Kühlbrandt 2021; Selstam and Sandelius 1984; Dehesh and Ryberg 1985; Lindsten et al. 1988). The atomic structure of LPOR assemblies resolved by electron cryo-microscopy reveals that LPOR polymerizes with Pchlide and NADPH into helical filaments around PLB lipid bilayer. *Arabidopsis* LPOR isoforms form helical filaments with lipids from the membranes of PLBs and chloroplasts. Here, the antiparallel LPOR dimers assemble into a strand. Portions of LPOR and Pchlide insert into the outer membrane leaflet, targeting the product, Chlide, to the membrane for the final reaction site of chlorophyll biosynthesis. In dark the LPOR filaments shape PLB membranes into high-curvature tubules. The light-induced disassembly of the PLB provides lipids for the organization of thylakoid membranes (Nguyen et al. 2021a, b; Solymosi and Mysliwa-Kurdziel 2021).

Subplastidic Chaperon-like protein of POR (CPP1) formerly Cdt1 (Cell growth factor 1) that contain J-like domain has been characterized in angiosperms such as *Arabidopsis*, *Nicotiana* (Lee et al. 2013) and *Gossypium* (Osborne et al. 2023). CPP1 helps in anchoring LPOR to PLBs, thus playing a crucial role in Chl synthesis and chloroplast biogenesis (Lee et al. 2013).

LPOR-Pchlide complexes -spectral properties

The Pchlide reduction reaction consists of 3 distinct steps including an initial light-driven step followed by dark steps which occur close to or above glass transition temp of proteins. The reduction reaction occurs at temperatures as low as 193 K, and in response to femtosecond manipulation of light pulses, signifying its biochemical novelty (Heyes and Hunter 2005, 2002; Heyes et al. 2006). Three spectrally different forms of Pchlide are formed at 77 K in etioplast due to the formation and aggregation of different sized enzyme ternary complex. F631 (due to the Pchlide structural arrangements), Pchlide F644 (mostly due to dimeric association of LPOR), and Pchlide F655 (due to oligomeric association with LPOR present in PLBs) (Sironval et al. 1965; Ryberg and Sundqvist 1982a; Böddi et al. 1989, 1990, 1998; Böddi and Frank 1997; Stadnichuk et al. 2005; Tripathy and Pattanayak 2011). F631 is the photochemically inactive or non-photoactive Pchlide that is not directly photoconvertible with a flash (Kósa et al. 2006).

These pigments are bound to the membrane surface of PTs in a monomeric form or bound to some protein other than LPOR or not present in the LPOR active site if bound to LPOR (Ryberg and Sundquist 1982a, b; Ikeuchi et al. 1983; Lindsten et al. 1988; Joyard et al. 1990; Solymosi and Mysliwa-Kurdziel 2021). The Pchlide component with emission at F644 are dimeric form or smaller oligomers of the LPOR ternary complex (Böddi 1991; Böddi et al. 1992, 1993; Martin et al. 1997; Chahdi et al. 1998). These dimers are located to the edge of the PLB membrane and they could be photo transformed with light of low intensity (Böddi 1991, 1992; Stadnichuk et al. 2005). Multimeric aggregate of the LPOR-dimers form F655 is the main photoactive form of Pchlide in etiolated plants that is transformed to Chlide (Böddi et al. 1989; Wiktorsson et al. 1993; Schoefs et al. 2000; Kósa et al. 2006). In these oligomeric POR–Chlide–NADPH ternary complexes Pchlide is bound to the active site of the LPOR macrodomain that are associated strongly with the tubular lamellae of PLBs (Ryberg and Sundquist 1982a; b; Solymosi and Schoefs 2008). These oligomeric complexes have a higher emission and are slowly dissociated into smaller complexes accompanied by the progressive release of Chlide from the LPOR catalytic site (Dalal and Tripathy 2012). Irradiation induces a series of changes in the ultrastructural and spectral properties of etioplasts that ultimately lead to the formation of chloroplasts.

Upon short illumination (30 s) Pchlide F655 is converted to Chlide F690 and subsequently to Chl (F682) (Litvin and Krasnovsky 1957; Franck and Mathis 1980; Böddi et al. 1993; Bodd̈di and Franck 1997; Lebedev and Timko 1999). Further illumination leads to the Chlide microcycle where the interconversion of oxidized and reduced forms of NADP proceeds. Ultimately, it leads to spectral blue shift (Shibata shift) at F680 nm (Shibata 1957). The kinetics of this shift is dependent on leaf age and environmental conditions (Shibata 1957; Dalal and Tripathy 2012). In intact leaves, a Shibata shift is usually completed within 10–30 min. Shibata shift is followed by the formation of photoactive photosystem II (PSII) units containing Chl F684 (Franck et al. 1999). The Shibata shift is arrested in extreme environmental conditions including water stress and heat stress resulting in impaired plastid development (Smeller et al. 2003; Dalal and Tripathy 2012; Mohanty and Tripathy 2011) Fig. 3.

LPOR protects plants from photooxidative damage

LPOR bestows photo-protection on the plants by limiting the Pchlide-mediated photo-oxidative damage (Buhr et al. 2008; Tripathy and Pattanayak 2012; Pattanayak and Tripathy 2011). Whereas the high light intensity on the surface of the ocean could photodamage slower LIPOR-containing

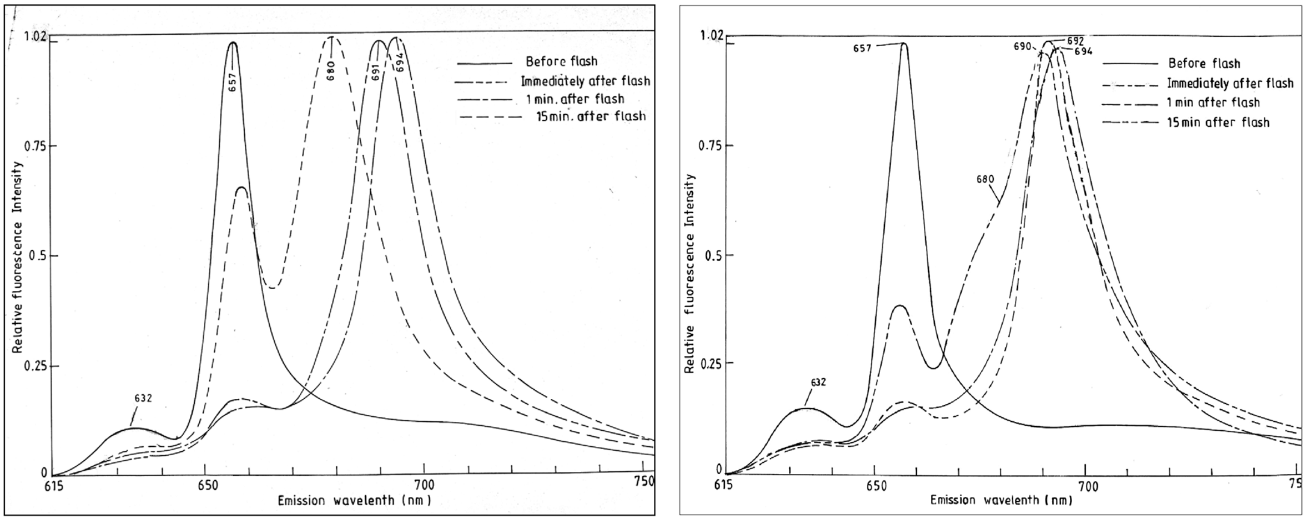

Fig. 3 Low temperature (77 K) fluorescence emission spectra (E440) of leaves from 6-day old etiolated control (upper panel) and water-stressed (lower panel) rice (PB1) seedlings, showing Shibata-shift. For water-stress, seedlings were treated with 50 mM PEG 6000, dissolved in nutrient solution, 16 h prior to taking spectra. Low temperature fluorescence emission spectra were recorded before flash, immediately after flash of 0.2 s and after 1 min and 10 min post-flash incubation (modified from Dalal and Tripathy 2012)

photoautotrophs, it can cause minimal damage to organisms possessing LPOR that converts the photosensitizer Pchlide to Chlide rapidly within 1 ms (Sytina et al. 2008; Soffe 2016; Heyes et al. 2021). LPOR protects the etiolated and green phototrophs by binding to the photosensitive Pchlide pool to keep it in photo-transformable form for very fast photoconversion of Pchlide to Chlide to minimize generation of $^1O_2$ that causes destruction of photosynthetic organisms in high light (Tripathy and Chakraborty 1991; Chakraborty and Tripathy 1992; Tripathy and Pattanayak 2011). Therefore, unlike LIPOR containing phototrophs, the LPOR containing organisms withstood the selection pressure of tetrapyrrole-photo-sensitized oxidative stress.

As the accumulation of porphyrins and Pchlide is toxic to plants as they act as photosensitizers to generate $^1O_2$ in light via type II photosensitization reaction. The $^1O_2$ causes severe damage to plants (Chakraborty and Tripathy 1992; Tripathy et al. 2007). The interruption of Chl synthesis during darkness requires suppression of the synthesis of 5-aminolevulinic acid (ALA), the first precursor molecule specific for Chl synthesis. The Pchlide and Chl biosynthesis is negatively regulated by FLU, a nuclear-encoded plastid protein. It mediates the regulatory effect by interacting with glutamyl-tRNA reductase (GluTR) to downregulate ALA biosynthesis in dark (Meskauskiene et al. 2001). The flu mutants have unregulated ALA and Pchlide biosynthesis that causes excess accumulation of Pchlide responsible for generation of $^1O_2$ that causes photooxidative damage via executor 1 and executor 2 (Meskauskiene et al. 2001; Wagner et al. 2004; Lee et al. 2007; Wang and Apel 2019). Conversely, FLU-overexpressing *Arabidopsis* lines suppress ALA synthesis resulting in reduced Chl content in light (Hou et al. 2019). Therefore, flu does not allow the synthesis of porphyrins and Pchlide in large amounts in plant tissues to prevent photooxidative damage. Binding of GluTR and LPOR to full-length FLU is essential for inhibiting ALA synthesis to avoid the overaccumulation of Pchlide in night (Hou et al. 2021). The FC2 isoform of heme catalysing enzyme ferrochelatase physically interacts with LPOR to stabilize the photoenzyme and suppress ALA synthesis to regulate Chl biosynthesis (Fan et al. 2023).

NADPH has several functions in the photoactive complexes. As a coenzyme, it provides the electrons and one proton for the reduction of Pchlide (Griffiths 1974). In etiolated tissues the LPOR forms a ternary complex with Pchlide and NADPH that aggregates into multimeric forms. After flash illumination NADPH photoreduces Pchlide to generate POR-NADP$^+$-Chlide complex. An immediate second flash is photooxidative as NADP$^+$ is incapable to photoreduce Pchlide. After few minutes of dark interval between the two flashes, the NADP$^+$ is re-reduced to NADPH that reduces Pchlide to Chlide. Thus, it is apparent that NADPH photo-protects the LPOR enzyme during early greening phase of angiosperms (Griffiths 1982; Franck and Inoue 1984).

Perspectives

Although we know the crystal structure of POR of certain prokaryotes, our knowledge of the structure of LPOR and its exact catalytic mechanism are still unclear in higher plants which often possess 3 different isoforms of the photo-enzyme. Besides, the reasons for the photo-lability and photo-stability of different isoforms LPOR are poorly understood. A comparative account of crystal structures of higher plant PORA, PORB and PORC and their catalytic mechanism shall be able to indicate the exact mechanism of catalysis and photo-stability. Overexpression of PORC protects plants from oxidative and other environmental stresses because of their evolution in stressful environment. This knowledge can be further exploited to raise crop plants tolerant to abiotic stresses.

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Declarations

Conflict of interest The authors declare that they have no known conflicting interest.

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